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(54) Title: RNA INTERFERENCE MEDIATING SMALL RNA MOLECULES

(57) Abstract: Double-stranded RNA (dsRNA) induces sequence-specific post-transcriptional gene silencing in many organisms by a process known as RNA interference (RNAi). Using a *Drosophila* *in vitro* system, we demonstrate that 19-23 nt short RNA fragments are the sequence-specific mediators of RNAi. The short interfering RNAs (siRNAs) are generated by an RNase III-like processing reaction from long dsRNA. Chemically synthesized siRNA duplexes with overhanging 3' ends mediate efficient target RNA cleavage in the lysate, and the cleavage site is located near the center of the region spanned by the guiding siRNA. Furthermore, we provide evidence that the direction of dsRNA processing determines whether sense or antisense target RNA can be cleaved by the produced siRNP complex.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 01/13968

A. CLASSIFICATION OF SUBJECT MATTER IPC 7 C12Q1/68 C12N15/10		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 7 C12Q C12N		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used) EPO-Internal, WPI Data, BIOSIS, MEDLINE, CHEM ABS Data		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>YANG DUN ET AL: "Evidence that processed small dsRNAs may mediate sequence-specific mRNA degradation during RNAi in Drosophila embryos."</p> <p>CURRENT BIOLOGY, vol. 10, no. 19, 19 September 2000 (2000-09-19), pages 1191-1200, XP001037751 ISSN: 0960-9822 the whole document</p> <p style="text-align: center;">-/-</p>	1-26
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex.		
* Special categories of cited documents : "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "&" document member of the same patent family		
Date of the actual completion of the international search 15 July 2003		Date of mailing of the international search report 29/07/2003
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016		Authorized officer Luzzatto, E

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 01/13968

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BASS BRENDA L: "Double-stranded RNA as a template for gene silencing." CELL, vol. 101, no. 3, 28 April 2000 (2000-04-28), pages 235-238, XP002194756 ISSN: 0092-8674 cited in the application the whole document	1-26
X	ZAMORE PHILLIP D ET AL: "RNAi: Double-stranded RNA directs the ATP-dependent cleavage of mRNA at 21 to 23 nucleotide intervals" CELL, CELL PRESS, CAMBRIDGE, MA, US, vol. 101, no. 1, 31 March 2000 (2000-03-31), pages 25-33, XP002208683 ISSN: 0092-8674 the whole document	1-29
A	TUSCHL THOMAS ET AL: "Targeted mRNA degradation by double-stranded RNA in vitro" GENES AND DEVELOPMENT, COLD SPRING HARBOR LABORATORY PRESS, NEW YORK, US, vol. 13, no. 24, 15 December 1999 (1999-12-15), pages 3191-3197, XP002183118 ISSN: 0890-9369 figures 3,5	16-18
A	HAMMOND SCOTT M ET AL: "An RNA-directed nuclease mediates post-transcriptional gene silencing in Drosophila cells" NATURE, MACMILLAN JOURNALS LTD. LONDON, GB, vol. 404, no. 6775, 16 March 2000 (2000-03-16), pages 293-296, XP002183123 ISSN: 0028-0836 the whole document	1-29
A	HAMILTON ANDREW J ET AL: "A species of small antisense RNA in posttranscriptional gene silencing in plants" SCIENCE, AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE, US, vol. 286, no. 5441, 29 October 1999 (1999-10-29), pages 950-952, XP002149064 ISSN: 0036-8075 cited in the application the whole document	1-29
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INTERNATIONAL SEARCH REPORT

International Application No.
PCT/EP 01/13968

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	ELBASHIR SAYDA M ET AL: "RNA interference is mediated by 21- and 22-nucleotide RNAs" GENES AND DEVELOPMENT, COLD SPRING HARBOR LABORATORY PRESS, NEW YORK, US, vol. 15, no. 2, 15 January 2001 (2001-01-15), pages 188-200, XP002204651 ISSN: 0890-9369 the whole document	1-29
P,X	WO 01 75164 A (MAX PLANCK GESELLSCHAFT ;TUSCHL THOMAS (DE); MASSACHUSETTS INSTITUTE) 11 October 2001 (2001-10-11) the whole document	1-29
A	DATABASE BIOSIS 'Online! BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; 1992 VINAYAK R ET AL: "CHEMICAL SYNTHESIS OF RNA USING FAST OLIGONUCLEOTIDE DEPROTECTION CHEMISTRY" Database accession no. PREV199294000583 XP002247596 abstract & NUCLEIC ACIDS RESEARCH, vol. 20, no. 6, 1992, pages 1265-1269, ISSN: 0305-1048	13,14

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INTERNATIONAL SEARCH REPORT

International application No.
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Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 1-12, 16-29 (all partly), 30-46 (completely)
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-12, 16-29 (all partly), 30-46 (completely)

1) Independent claims 1 and 16 relate to "target-specific nucleic acid modifications".

However, the description only provides data as to RNA interference: neither for DNA methylation nor for any other "target specific nucleic acid modifications" are provided data of any kind. Hence, claims 1 and 16 and claims 2-11 and 17-19 dependent thereon lack support, insofar as not limited to RNA interference (Art. 6 PCT). The same applies to claims 20-26, related to the use of the method of claims 16-19.

2) Product claims 1-12 relate to double stranded RNA molecules having a length of between 19 and 25 nt solely characterised in that they mediate RNA interference. This sole functional feature, however, is not sufficient to characterise the claimed molecules so as to allow the skilled person to clearly and unambiguously understand the scope of the claims. Moreover, the skilled person is given no guidance, either in the claims or in the description, as to any general technical feature of the claimed RNA molecules which could allow him/her to understand, with no undue burden, which molecules fall and which do not fall within the definition of the claims.

3) Thus, claims 1-12 lack clarity and support (Art. 6 PCT) to such an extent as to render a meaningful search with respect to the whole breadth of these claims impossible. The search with respect to their subject-matter (as well as to that of claims 27-29, which relate to compositions comprising the RNA molecules of claims 1-12) has thus been limited to the use of ds RNA molecules having a length of 19-25 nt and compositions comprising them for RNA interference-based methods, whereas the search with respect to claims 16-26 has been only carried out insofar as related to RNA interference.

In view of the objections set out in items 1 and 2 hereinabove, claims 13-15 have been searched with respect to a method for the preparation of ds RNA molecules in general characterised by steps a) and b) of claim 13.

4) Claim 30 relates to a knockout cell obtained by transfecting it with at least one ds RNA molecule capable of inhibiting the expression of an endogenous gene. However, the sole indication that a cell has been obtained by treating it with a short RNA molecule with no indication as to features of the cell, such as e.g. the kind of cell and the kind of gene which has been silenced does not allow the skilled person to clearly and unambiguously understand the scope of the claim.

Moreover, although the description mentions possible knockout phenotypes (p. 10), it falls short of providing any examples or experimental data as to such a knockout cell.

Claim 30 lacks thus clarity and support (Art. 6 PCT) to such an extent as to render a meaningful search of its subject-matter impossible.

The same applies to claims 31-44 which are directly or indirectly dependent on claim 30.

Claims 30-44 thus have not been searched.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

5) The same arguments concerning lack of clarity set out for claims 1-12 apply to claims 45-46, which relate to ds RNA molecules capable of inhibiting a target gene. Moreover, although the application mentions methods for identifying and/or characterising pharmacological agents (p. 12, l. 19-23), it does not however provide any concrete indication or experimental data as to such methods, in particular as to which pharmacologically relevant genes could be targeted by a method for identifying pharmacological compounds based on RNA interference. Hence claims 45-46 lack clarity and support (Art. 6 PCT) to such an extent as to render a meaningful search of their subject-matter impossible.

No search has thus been carried out with respect to these claims.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 01/13968

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 0175164	A	11-10-2001	AU 3574402 A	11-06-2002
			AU 4962201 A	15-10-2001
			CA 2404890 A1	11-10-2001
			WO 0244321 A2	06-06-2002
			EP 1309726 A2	14-05-2003
			WO 0175164 A2	11-10-2001
			US 2003108923 A1	12-06-2003
			US 2002086356 A1	04-07-2002

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